Prevalence of Birefringent Crystals in Cardiac and Prostatic Tissues

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Title: Prevalence of Birefringent Crystals in Cardiac and Prostatic Tissues

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I. Abstract

Introduction: The prevalence of urate crystals in residual tissue samples from coronary arteries, aortic valves and prostate glands was assessed.

Methods: Alcohol-fixed coronary arteries from 55 explanted hearts, alcohol-fixed aortic valves collected from 75 valve replacement surgeries, and 40 frozen, unfixed prostate specimens resected during cancer surgery were examined for birefringent crystals with polarizing microscopy.

Results: In the 55 explanted hearts, 6 (10.9%) contained a coronary artery with birefringent crystals. One of the 75 aortic valves (1.4%) contained both negatively and positively birefringent crystals. 19 of the 40 (47.5%) prostates contained birefringent crystals.

Conclusion: We found that a remarkable percentage of coronary arteries and prostate specimens contained birefringent crystals. Crystal presence is an obvious prerequisite for possible crystal induced inflammation in these tissues, just as similar crystals elicit a gouty inflammatory cascade in synovial joints. Further studies are necessary to determine whether urate crystals may play this role in these tissues, and if so, to establish whether urate-lowering therapy may be beneficial in prostatitic and coronary disease.

Strengths:

• First study to systematically evaluate for the prevalence of urate crystals within tissues.

• First study to show a significant percentage of extra-articular negatively birefringent crystals.

Limitations:

- Secondary methods, aside from polarizing microscopy, to confirm urate composition of

the visualized crystals were unsuccessful.

- Clinical correlations were not possible for coronary and prostate samples since these were

taken from anonymous repositories collected for non-rheumatic diseases.

II. INTRODUCTION

By definition, hyperuricemic interstitial fluids are supersaturated, and urate supersaturation leads to precipitation of urate crystals. In joints, the crystals often drive the inflammatory disease recognized as gouty arthritis. However, hyperuricemia is not limited to plasma and the joints. All other interstitial fluids (excepting cerebrospinal fluid and sweat) will be supersaturated as well. Consequently, urate crystals will occur in non-articular tissues and may well drive crystal-induced inflammation.

In fact, anecdotal observations of visible and/or palpable tophaceous deposits have been reported from many different organs,[1] but they have usually been considered to be "medical oddities."[2] Newer imaging modalities of ultrasound and dual-energy computed tomography (DECT) have revealed far more extensive peri-articular deposition than we previously suspected.[3-5] To date, however, we have limited microscopic evidence of extra-articular urate deposition, and we do not know whether the published anecdotes are unique. As an initial venture into the field of crystal prevalence, we obtained access to unselected tissue specimens from coronary arteries, cardiac valves, and prostate glands. At each of these sites, crystal-driven inflammation could play an important role in the local pathophysiology.

Gout and hyperuricemia have been associated with hypertension, coronary artery disease, and all-cause mortality,[6-13] but the mechanisms responsible for these associations remain unclear. Atherosclerotic disease is a ubiquitous process involving plaque formation throughout the arterial tree. The formation of these lesions is now seen as a process of lipid deposition within

the intima of the vessel wall, oxidation of the lipoproteins, followed by endothelial activation - resulting in leukocyte adhesion and migration into the vessel wall.[14-16] The presumed pathways are complex, involving T cells, macrophages, neutrophils and dendritic cells, accumulation of apoptotic cells, and ultimately to plaque formation, rupture and thrombosis.[17-19]

We suggest that urate crystals in the arterial intima of gouty patients could, in some cases, drive the inflammation that causes plaques to rupture, and could thereby factor in their cardiac risk. This role is well established in gouty joints where microcrystalline activation of the NALP3 inflammasome pathway induces multiple inflammatory mediators including IL-1β,[20] and leads to gouty arthritis. In sum, MSU crystal deposits within coronary arteries could potentially trigger a corresponding inflammatory cascade that eventuates in thrombosis and infarction.

We are aware of only one previous report of apparent urate crystals in a coronary artery. There, Traut, et al[21] describe a man with tophaceous gout who died of a ruptured abdominal aortic aneurysm with birefringent, spherulitic deposits in the intima of a coronary artery on autopsy. This report preceded the application of first order red plates,[22] so it is not known whether the crystalline deposits were negatively birefringent, as urate crystals are, or positively birefringent, as calcium pyrophosphate crystals are.

In contrast to the rarity of arterial crystals, multiple reports of cardiac valvular tophi have been published.[23-28] Presumably, individual MSU crystals must also deposit on the valves, though their presence has not been described. Aortic stenosis is a common cause of valvular disease,

with calcific stenosis prevalence increasing with age to approximately 2.8% in ages ≥75 years.[29]

Aortic valve degeneration is also associated with male gender, hypertension, and metabolic

syndrome,[30,31] all risk factors associated with hyperuricemia and gout. Additionally, higher

serum uric acid levels have been described in patients with severe calcific aortic stenosis

compared to mild or moderate aortic stenosis.[32]

Lastly, chronic nonbacterial prostatitis or chronic pelvic pain syndrome (CPPS), a condition

characterized by chronic pelvic pain of unidentifiable cause for greater than three of the

preceding six months, is a common problem, affecting 2-10% of men.[33] Effective treatment

remains elusive, and the condition is frustrating to physicians as well as affected patients.

Various etiologies have been proposed, including occult infection, neurologic dysfunction,

psychological factors, and immune dysregulation.[34] The role of inflammation remains

controversial, as the presence of leukocytes on prostate biopsy does not correlate with severity

of symptoms.[35] However, molecular studies have shown increased cytokine levels in CPPS

patients, including IL-8, CXCL5 or epithelial neutrophil activating peptide 78 (ENA-78),[36] tumor

necrosis factor α (TNFα), and interestingly IL-1β.[37] In addition, a study of expressed prostatic

secretions in 56 men with chronic prostatitis found that higher urate concentrations correlated

with prostatitic symptoms, though the specimens were not examined for crystals.[38] The

authors then performed a placebo-controlled trial of allopurinol at 300mg or 600mg a day in 54

men for 8 months. The allopurinol treated patients demonstrated statistically significant lower

urate levels in expressed prostatic secretions as well as improved subjective pain compared to

the placebo group.[39] To our knowledge, MSU crystals have not been sought or identified in

prostate tissue prior to our present study.

Given the evidence that tophaceous MSU deposition is widespread and that hyperuricemia now

approaches epidemic proportions, we suspected that subclinical, microscopic deposition must

also be widespread. To address this suspicion, we carried out a pilot crystal prevalence study in

3 human conditions where appropriate tissue samples were available and chronic inflammation

remains problematic: coronary disease, aortic stenosis, and chronic prostatitis.

III. METHODS

Coronary artery evaluation. Coronary arteries (left anterior descending, right coronary, left

main, and circumflex) fixed in methacarn [60% absolute methanol (Sigma-Aldrich, St. Louis,

MO), 30% chloroform (Sigma-Aldrich, St. Louis, MO), 10% glacial acetic acid (Sigma-Aldrich, St.

Louis, MO)] obtained from fifty-five explanted hearts at the time of heart transplant conducted

from 2000-2003 at the University of Washington Medical Center were examined with polarizing

microscopy (Olympus BX50, Tokyo, Japan) and photographed (Canon EOS 5D Mark II, Tokyo,

Japan). Three to six consecutive sections of each artery were examined. To be considered

positive, visualized crystals had to be present in two or more sections the same specimen.

Deconvolution microscopy (Zeiss 510 META, Oberkochen, Germany) was used to verify the

intracellular nature of the crystals in one specimen. Clinical records of these individual patients

were not available as the specimens had been de-identified as required by the University of

Washington institutional review board. Since the clinical indications for cardiac transplantation

have not changed, we carried out a demographic review of 50 consecutive patients undergoing

heart transplantation from January 2010-March 2012 (Table 1). Of these fifty patients, 19

(38%) had a history of gout and 24 (48%) were hyperuricemic (serum uric acid > 7mg/dL).

Table 1. Clinical characteristics of heart transplant patients from 2010-2012. Coronary arteries

for this study were collected from heart transplants occurring from 2000-2003.

	N	%
Gender		
Males	40	80
Females	10	20
Age (years)		
19-29	4	8
30-39	2	4
40-49	10	20
50-59	20	40
60-69	14	28
>70	0	0
Gout	19	38
Hyperuricemia (serum uric acid >7mg/dL)	24	48
Urate lowering therapy	16	32
Diuretic use	34	68
Chronic Kidney Disease	30	60
Indication for transplant		
Ischemic cardiomyopathy	19	38
Dilated cardiomyopathy	18	36
Other	13	26

Aortic valve evaluation. Seventy-five aortic valves obtained from aortic valve replacement

conducted from 2011-2013 were fixed in methacarn, decalcified in RDO acid (Apex Engineering,

Plainfield, IL), and examined with polarizing microscopy. Four consecutive sections were

examined for each valve. To be considered positive, visualized crystals had to be present in two

or more sections of the same specimen. Clinical information for these patients was available

for review though serum urate was known for only four subjects. Eight of the 75 (10.7%)

patients had a history of gout, 6 (8%) were on allopurinol, 31 (41.3%) were on diuretics, 17

(22.7%) with renal insufficiency. Three patients (4.0%) had a SUA > 7mg/dL, and all three of

these patients also carried a diagnosis of gout.

Prostate evaluation. Forty frozen, unfixed, prostates from consecutive radical prostatectomies

performed for prostate cancer from 2012-2013 were assessed. Non-malignant regions were

evaluated with polarizing microscopy. One section from each prostate was examined. H&E

stained serial sections were examined independently for inflammatory foci in a blinded fashion.

Inflammation was graded on a scale of I-IV based on number of inflammatory foci consisting of

mononuclear infiltrates, giant cells, and/or polymorphonuclear cells. Grade I was assigned for

1-2 foci, grade II for 3-4 foci, grade III for 4-5 foci, and grade IV for 6 or more foci of

inflammatory cells within the section.

None of the patients had a recorded history of chronic prostatitis, gout, urate-lowering therapy,

or hyperuricemia.

The University of Washington Institutional Review Board approved our crystal-prevalence

study.

IV. RESULTS

Coronary artery evaluation. Of the 55 hearts examined, 6 arteries from different hearts (10.9%), contained birefringent crystals. One patient had intracellular, negatively birefringent, acicular crystals in the intima of the left anterior descending (Figure 1). One patient had a microtophus in the intima of the left anterior descending (Figure 2). One patient had negatively birefringent crystals and microtophi in the adventitia of the right coronary artery (Figure 3). Three additional patients had highly birefringent accumulations of intimal, extracellular unidentifiable crystals.

Aortic valve evaluation. Of the 75 aortic valves assessed, one valve (1.3%) had both positively and negatively birefringent crystals present scattered throughout. The visualized crystals were present in two or more sections of the same specimen.

Prostate evaluation. Nineteen of the forty prostates (47.5%) contained abundant, acicular (needle-shaped) birefringent crystals. The majority were negatively birefringent, though positively birefringent crystals were also seen. All crystals were present within the lumens of the glands (Figure 4).

By severity of inflammation, crystals were seen in 5 of the 12 grade I specimens, 6 of 13 grade II specimens, 4 of 6 grade III specimens, 4 of 7 grade IV specimens. Thirty-eight specimens contained mononuclear infiltrates, nine with polymorphonuclear cells, and one with giant cells. Thus, presence of crystals did not correlate with inflammatory foci grade.

V. CONCLUSIONS

When serum is hyperuricemic, so too are all other extracellular body fluids other than cerebrospinal fluid and sweat. Urate crystals are known to precipitate in loci other than the joints when supersaturated with uric acid. Anecdotal tophi have been reported in many different organs[1] though prevalence data of individual crystals are lacking. Are such deposits exceptional, or are they the tip of a crystalline iceberg?

Little data exists on the prevalence of tissue MSU crystal deposition in non-gouty patients. An autopsy study of 1733 unselected Australian patients found 8% of kidneys contained urate deposits,[40] many from individuals with no known history of gout. In classic autopsy studies of 279 gouty subjects, Talbott found normal kidneys in only four, and gross crystalline deposits in many.[41] In others, crystal presence could be ascertained by the gritty feeling when the kidneys were cut with a knife.[2] A study of cadaveric first metatarsophalangeal joints found 17% of 70 consecutive autopsies of men and women contained MSU crystals. Only 2 of the 12 patients with MSU crystals had a history of podagra or gout.[42] With the advent of newer imaging modalities such as ultrasound and dual energy computed tomography (DECT), our understanding of urate deposition continues to evolve,[3-5] but we know of no other studies of microscopic prevalence.

Our study demonstrates crystal deposition within a significant percentage of coronary arteries with one artery containing characteristic acicular, intracellular negatively birefringent crystals.

In that specimen, one focus of mononuclear cells contained many with crystalline inclusions, but no polymorphonuclear leukocytes were seen. Validation of our hypothesis, that crystal-driven inflammation may cause plaque rupture, and coronary thrombosis in some gouty patients, would seem to require intracellular crystals at the site of such a fatal lesion. To make that observation, the pathologist must have sufficient suspicion to fix the tissue in alcohol, due to formalin's formation of highly soluble addition products with urate,[43] and must then examine the resultant sections under polarized light. Neither alcohol fixation nor polarized inspection are standard procedures, but we hope that pathologists evaluating lethal myocardial infarctions would be willing to use them in search of this critically important possibility.

Confirmation of crystal composition can be difficult in tissue sections. In contrast to the thin film seen by rheumatologists in synovial aspirates, most tissue slices are ~7 microns in thickness and this is about the length of the prototypic urate crystal. Most crystals will then angle away from the tissue plane and appear to be short with varying thickness. In addition, the cytoplasmic constituents above and below a crystal of interest will lead to light scattering with indistinct crystal borders. We suspect that these issues are the principal reasons why most of the reports of crystal deposits in nonarticular locations are not illustrated by a photograph.

We further suspect that there is an as yet undefined alteration in the composition of urate deposits in some tissues. In some of the microtophi we have observed, the crystals occur not only in the classic needle and/or spherulite form but also as plates which sometimes stack upon each other. Plate-like crystals have been described in tophi in addition to classic needle-shaped

crystals in a case report.[44]   Additionally, Spitz, et al in 1949 observed two morphologic types of

crystals in bony, cartilage, and renal tophi in a man with diffuse tophaceous gout.[45] Crystals

with "fine needles" in a "radial arrangement" with surrounding giant cells, lymphocytes and

fibroblasts were described. These typical crystals dissolved with formalin fixation, paraffin

embedding, and H&E staining but did not dissolve with alcohol fixation and De Galantha

staining. A second type of crystal remained after formalin fixation, paraffin embedding, H&E

staining, and decalcification with weak acids (sodium citrate and formic acid). These were

described as rhomboid, "doubly refractive," with "longitudinal striations," and oriented in

"irregular clusters or sheaves of elongated blunt needles." We have studied such deposits in

slides from the kidney of a man with severe, tophaceous gout. The slides were decalcified so

calcium crystals were unlikely. The deposits did not dissolve with topical uricase or formalin.

They did not appear to be protein-coated since applied protease did not affect their resistance

to uricase. They did stain positively with De Galantha's stain, though this classic stain for urate

crystals can be nonspecific.[46] We mention this experience because the long-standing

hyperuricemia and abundant tophi in this man with a long term history of repeated gouty

arthritis made that diagnosis undeniable. We acknowledge that not all of the crystals we have

found must be monosodium urate monohydrate. Urates aside from monosodium urate,

including calcium urate, potassium urate, and magnesium urate, are known to form within the

urinary tract leading to renal calculi,[47,48] and could be contributing to this other type of crystal

seen in renal tophi and other tissues as well.

The great majority of the crystals we found in prostates were morphologically identical to those

we expect to see in gouty joints. Although none of the prostates we examined were obtained

from patients with a recognized history of gout or hyperuricemia, we believe this may not be

accurate. Most of these men were referred to our urologists from outside of our hospital

system, and the exact prevalence of gout and hyperuricemia may not have been reflected in

the medical record.

We chose to study prostates because they are so often afflicted with chronic inflammation, and

we had access to a large repository of prostate tissues. Each specimen was frozen and thus had

not seen the formalin fixation that dissolves most tissue urate before the pathologist ever sees

the slides. All of these specimens also contained malignant areas, but no control prostates

were examined. Our data cannot be taken as indicative of a causal link between crystals and

cancer.

A potential mechanism of prostatic crystal deposition could be due to reflux of urine

supersaturated with uric acid. However, the crystal morphology we observed was clearly that

of urate rather than uric acid. Irrespective of its source, the presence of so many urate crystals

strongly suggests the possibility that some, much, or most prostatitis is crystal-driven. Given

the highly effective armamentarium with which we now fight gout, the investigational

opportunities are obvious as is the hope that such medications may be found of use in the

management of this frustrating problem.

Only a small percentage of aortic valves in this study contained birefringent crystals; thus

reinforcing the concept that most of the apparently "tophaceous" deposits at this site are

calcific. MSU crystals are unlikely to be a significant contributor to aortic valve disease, but the

possibility continues to merit consideration in the hyperuricemic patient with tophi elsewhere.

Over the years the conception of gout has evolved away from that of a periodic nuisance that

sometimes could be damaging. Instead, it is increasingly clear that this is a progressive,

systemic disease with panoply of injurious manifestations throughout the body. We hope that

our small pilot study will encourage others to examine the epidemiology of urate deposition as

well as the consequences of such deposits throughout the body. Uric acid itself has been

recognized as a damage-associated molecular pattern (DAMP),[49,50] and there has been

significant debate about whether uric acid is pathologic or a bystander to tissue injury. In this

study we found that MSU crystals are present in coronary arteries and prostates, suggestive

that they may play a role as a trigger for the NALP3 inflammasome pathway, just as they do

within the joint. Further studies are warranted to investigate the consequences to MSU crystals

within these tissues.

Contributorship Statement: JP performed polarizing microscopy, chart review, and drafted the

manuscript. MR provided prostate pathology expertise. DS collected tissue samples and

performed polarizing microscopy. NM provided cardiac tissue samples. PS conceived and

designed the study, and drafted the manuscript. All authors read and approved the final

manuscript.

Data Sharing Statement: There are several photos of crystals within prostates that were not

included. Photos of the crystals of the one aortic valve were not included. Photos are available

by emailing janepark@uw.edu.

References:

1.   Forbess LJ, Fields TR. The broad spectrum of urate crystal deposition: unusual presentations of gouty tophi. *Semin Arthritis Rheum*. 2012;**42**:146-54.

2.   Weiss TE, Segaloff A. Gouty arthritis and gout; an ancient disease with modern interest. Springfield, Ill.,: Thomas; 1959.

3.   Dalbeth N, Kalluru R, Aati O, et al. Tendon involvement in the feet of patients with gout: a dual-energy CT study. *Ann Rheum Dis*. 2013;**72**:1545-8.

4.   Gruber M, Bodner G, Rath E, et al. Dual-energy computed tomography compared with ultrasound in the diagnosis of gout. *Rheumatology (Oxford)*. 2013;**72**:1545-1548.

5.   McQueen FM, Reeves Q, Dalbeth N. New insights into an old disease: advanced imaging in the diagnosis and management of gout. *Postgrad Med J*. 2013;**89**:87-93.

6.   Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med*. 2008;**359**:1811-21.

7.   Ioachimescu AG, Brennan DM, Hoar BM, et al. Serum uric acid is an independent predictor of all-cause mortality in patients at high risk of cardiovascular disease: a preventive cardiology information system (PreCIS) database cohort study. *Arthritis Rheum*. 2008;**58**:623-30.

8.   Kuo CF, See LC, Luo SF, et al. Gout: an independent risk factor for all-cause and cardiovascular mortality. *Rheumatology (Oxford)*. 2010;**49**:141-6.

9.   Moriarity JT, Folsom AR, Iribarren C, et al. Serum uric acid and risk of coronary heart disease: Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol*. 2000;**10**:136-43.

10.   Krishnan E, Pandya BJ, Chung L, et al. Hyperuricemia and the risk for subclinical coronary atherosclerosis--data from a prospective observational cohort study. *Arthritis Res Ther*. 2011;**13**:R66.

11.   Choi HK, Curhan G. Independent impact of gout on mortality and risk for coronary heart disease. *Circulation*. 2007;**116**:894-900.

12.   De Vera MA, Rahman MM, Bhole V, et al. Independent impact of gout on the risk of acute myocardial infarction among elderly women: a population-based study. *Ann Rheum Dis*. 2010;**69**:1162-4.

13.   Chen JH, Chuang SY, Chen HJ, et al. Serum uric acid level as an independent risk factor for all-cause, cardiovascular, and ischemic stroke mortality: a Chinese cohort study. *Arthritis Rheum*. 2009;**61**:225-32.

14.   Lichtman AH, Binder CJ, Tsimikas S, et al. Adaptive immunity in atherogenesis: new insights and therapeutic approaches. *J Clin Invest*. 2013;**123**:27-36.

15.   Stoll G, Bendszus M. Inflammation and atherosclerosis: novel insights into plaque formation and destabilization. *Stroke*. 2006;**37**:1923-32.

16.   Legein B, Temmerman L, Biessen EA, et al. Inflammation and immune system interactions in atherosclerosis. *Cell Mol Life Sci*. 2013;**70**:3847-69.

17.   Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell*. 2011;**145**:341-55.

18.   Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med*. 2011;**17**:1410-22.

19.   Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol*. 2011;**12**:204-12.

20.   Martinon F, Petrilli V, Mayor A, et al. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*. 2006;**440**:237-41.

21.   Traut EF, Knight AA, Szanto PB, et al. Specific vascular changes in gout. *JAMA*. 1954;**156**:591-3.

22.   McCarty DJ, Hollander JL. Identification of urate crystals in gouty synovial fluid. *Ann Intern Med*. 1961;**54**:452-60.

23. Dennstedt FE, Weilbaecher DG. Tophaceous mitral value: Report of a case. *Am J Surg Pathol*. 1982;**6**:79-81.

24. Jaworski RC, Gibson M. Tophaceous aortic valve: a case report. *Pathology*. 1983;**15**:197-9.

25. Curtiss EI, Miller TR, Shapiro LS. Pulmonic regurgitation due to valvular tophi. *Circulation*. 1983;**67**:699-701.

26. Scalapino JN, Edwards WD, Steckelberg JM, et al. Mitral stenosis associated with valvular tophi. *Mayo Clin Proc*. 1984;**59**:509-12.

27. Gawoski JM, Balogh K, Landis WJ. Aortic valvular tophus: identification by X-ray diffraction of urate and calcium phosphates. *J Clin Pathol*. 1985;**38**:873-6.

28. Iacobellis G. A rare and asymptomatic case of mitral valve tophus associated with severe gouty tophaceous arthritis. *J Endocrinol Invest*. 2004;**27**:965-6.

29. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation*. 2013;**127**:e6-e245.

30. Stewart BF, Siscovick D, Lind BK, et al. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. *J Am Coll Cardiol*. 1997;**29**:630-4.

31. Katz R, Wong ND, Kronmal R, et al. Features of the metabolic syndrome and diabetes mellitus as predictors of aortic valve calcification in the Multi-Ethnic Study of Atherosclerosis. *Circulation*. 2006;**113**:2113-9.

32. Demir B, Caglar IM, Ugurlucan M, et al. The relationship between severity of calcific aortic stenosis and serum uric acid levels. *Angiology*. 2012;**63**:603-8.

33. Krieger JN, Riley DE, Cheah PY, et al. Epidemiology of prostatitis: new evidence for a world-wide problem. *World J Urol*. 2003;**21**:70-4.

34. Pontari MA. Chronic prostatitis/chronic pelvic pain syndrome. *Urol Clin North Am*. 2008;**35**:81-9; vi.

35. True LD, Berger RE, Rothman I, et al. Prostate histopathology and the chronic prostatitis/chronic pelvic pain syndrome: a prospective biopsy study. *J Urol*. 1999;**162**:2014-8.

36. Hochreiter WW, Nadler RB, Koch AE, et al. Evaluation of the cytokines interleukin 8 and epithelial neutrophil activating peptide 78 as indicators of inflammation in prostatic secretions. *Urology*. 2000;**56**:1025-9.

37. Nadler RB, Koch AE, Calhoun EA, et al. IL-1beta and TNF-alpha in prostatic secretions are indicators in the evaluation of men with chronic prostatitis. *J Urol*. 2000;**164**:214-8.

38. Persson BE, Ronquist G. Evidence for a mechanistic association between nonbacterial prostatitis and levels of urate and creatinine in expressed prostatic secretion. *J Urol*. 1996;**155**:958-60.

39. Persson BE, Ronquist G, Ekblom M. Ameliorative effect of allopurinol on nonbacterial prostatitis: a parallel double-blind controlled study. *J Urol*. 1996;**155**:961-4.

40. Linnane JW, Burry AF, Emmerson BT. Urate deposits in the renal medulla. Prevalence and associations. *Nephron*. 1981;**29**:216-22.

41. Talbott JH. Gout. New York, NY: Grune & Stratton; 1957.

42. Wall B, Agudelo CA, Tesser JR, et al. An autopsy study of the prevalence of monosodium urate and calcium pyrophosphate dihydrate crystal deposition in first metatarsophalangeal joints. *Arthritis Rheum*. 1983;**26**:1522-4.

43. Simkin PA, Bassett JE, Lee QP. Not water, but formalin, dissolves urate crystals in tophaceous tissue samples. *J Rheum*. 1994;**21**:2320-1.

44. Sen B, Arora VK, Gupta K, et al. Platelike urate crystals in gouty tophi. **Acta Cytol**. 1993;**37**:640-1.

45. Spitz H, Steinbrocker O. Fulminating fatal gout. *Am J Med*. 1949;**6**:513-21.

46. Shidham V, Shidham G. Staining method to demonstrate urate crystals in formalin-fixed, paraffin-embedded tissue sections. *Arch Pathol Lab Med*. 2000;**124**:774-6.

47. Herring LC. Observations on the analysis of ten thousand urinary calculi. *J Urol*. 1962;**88**:545-62.

48.   Bellanato J, Cifuentes JL, Salvador E, et al. Urates in uric acid renal calculi. *Int J Urol*. 2009;**16**: 318-21; discussion 22.

49.   Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature*. 2003;**425**:516-21.

50.   Kono H, Chen CJ, Ontiveros F, et al. Uric acid promotes an acute inflammatory response to sterile cell death in mice. *J Clin Invest*. 2010;**120**:1939-49.

Figure Legends:

Figure 1. Intracellular negatively birefringent crystals in the intima of a left anterior descending

artery (left) as seen with polarizing microscopy. White arrow denotes first-order red plate axis.

Deconvolution confocal microscopy image of the same cell showing intracellular crystals (right).

Figure 2. Microtophus in the intima of a left anterior descending artery.

Figure 3. Microtophi in the adventitia of a right coronary artery.

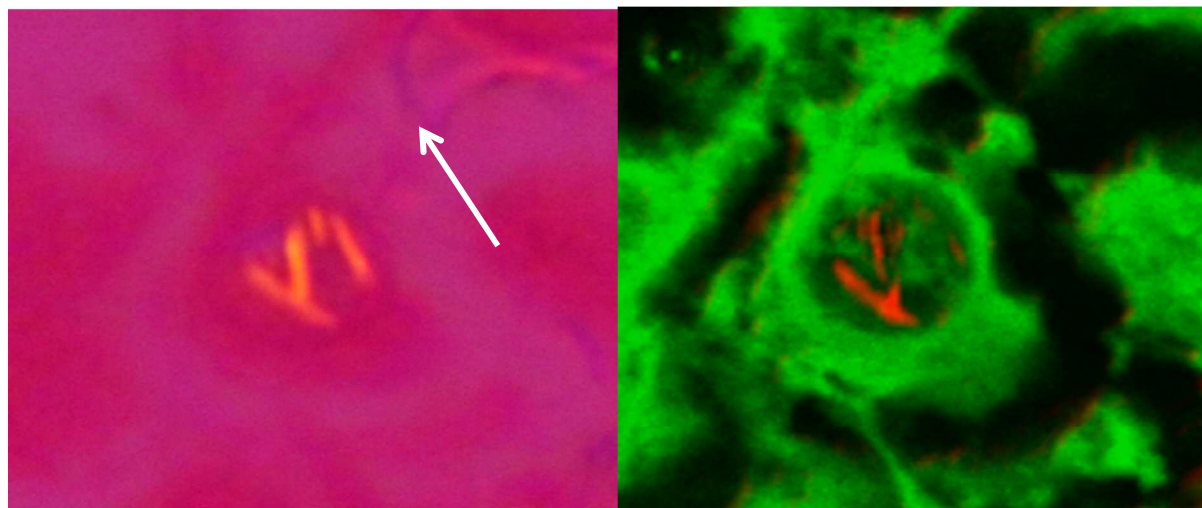**Figure 4**. Positively and negatively birefringent crystals within prostate gland lumen.
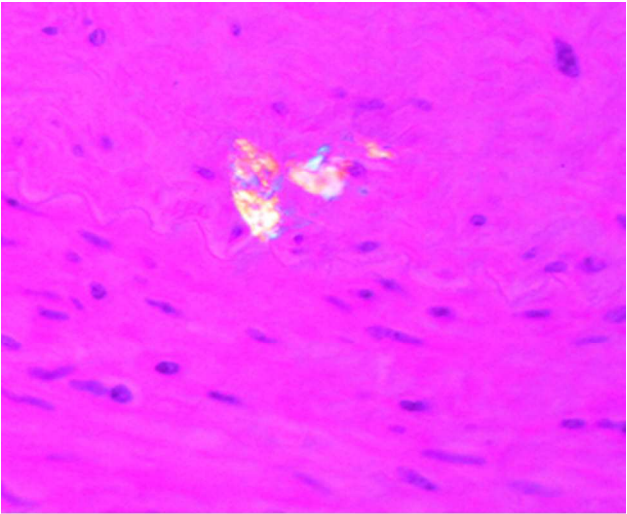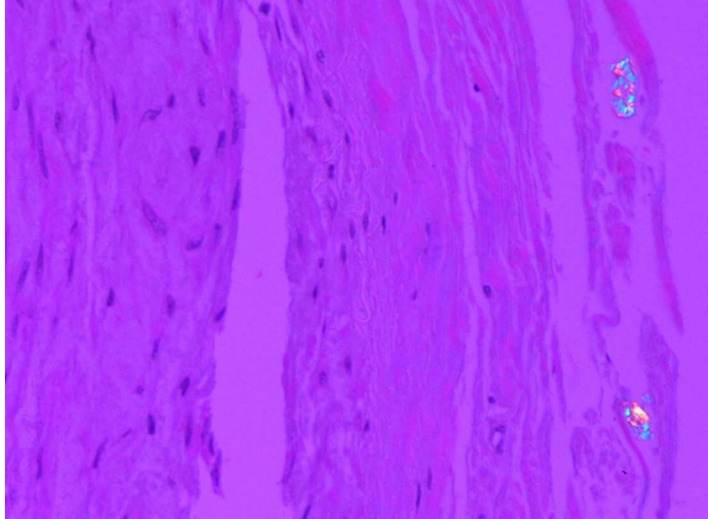
Figure 1.

Figure 2.

Figure 3.

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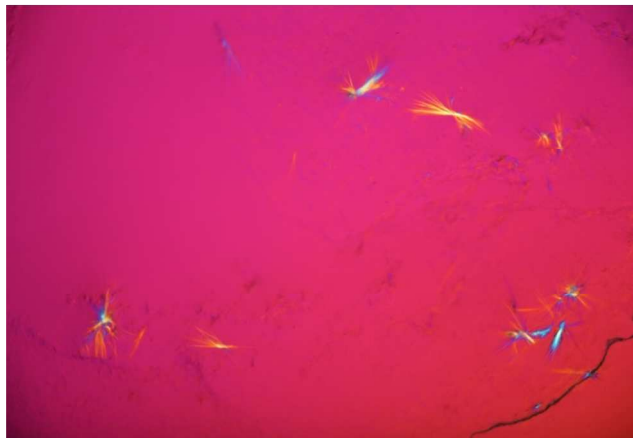39
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Figure 4.

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Prevalence of Birefringent Crystals in Cardiac and Prostatic Tissues, an Observational Study

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Prevalence of Birefringent Crystals

Title: Prevalence of Birefringent Crystals in Cardiac and Prostatic Tissues, an Observational

Study

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I. Abstract

Introduction: The prevalence of urate crystals in residual tissue samples from coronary arteries, aortic valves and prostate glands was assessed.

Methods: Alcohol-fixed coronary arteries from 55 explanted hearts, alcohol-fixed aortic valves collected from 75 valve replacement surgeries, and 40 frozen, unfixed prostate specimens resected during cancer surgery were examined for birefringent crystals with polarizing microscopy.

Results: In the 55 explanted hearts, 6 (10.9%) contained a coronary artery with birefringent crystals. One of the 75 aortic valves (1.4%) contained both negatively and positively birefringent crystals. 19 of the 40 (47.5%) prostates contained birefringent crystals.

Conclusion: We found that a remarkable percentage of coronary arteries and prostate specimens contained birefringent crystals. Crystal presence is an obvious prerequisite for possible crystal induced inflammation in these tissues, just as similar crystals elicit a gouty inflammatory cascade in synovial joints. Further studies are necessary to determine whether urate crystals may play this role in these tissues, and if so, to establish whether urate-lowering therapy may be beneficial in prostatitic and coronary disease.

Strengths:

• First study to systematically evaluate for the prevalence of urate crystals within tissues.

• First study to show a significant percentage of extra-articular negatively birefringent crystals.

Limitations:

- Secondary methods, aside from polarizing microscopy, to confirm urate composition of

the visualized crystals were unsuccessful.

- Clinical correlations were not possible for coronary and prostate samples since these were

taconary from anonymous repositories collected for non-rheumatic diseases.

II. INTRODUCTION

By definition, hyperuricemic interstitial fluids are supersaturated, and urate supersaturation

leads to precipitation of urate crystals. In joints, the crystals often drive the inflammatory

disease recognized as gouty arthritis. However, hyperuricemia is not limited to plasma and the

joints. All other interstitial fluids (excepting cerebrospinal fluid and sweat) will be

supersaturated as well. Consequently, urate crystals will occur in non-articular tissues and may

well drive crystal-induced inflammation.

In fact, anecdotal observations of visible and/or palpable tophaceous deposits have been

reported from many different organs,[1] but they have usually been considered to be "medical

oddities."[2] Newer imaging modalities of ultrasound and dual-energy computed tomography

(DECT) have revealed far more extensive peri-articular deposition than we previously

suspected.[3-5] To date, however, we have limited microscopic evidence of extra-articular urate

deposition, and we do not know whether the published anecdotes are unique. As an initial

venture into the field of crystal prevalence, we obtained access to unselected tissue specimens

from coronary arteries, cardiac valves, and prostate glands. At each of these sites, crystal-

driven inflammation could play an important role in the local pathophysiology.

Gout and hyperuricemia have been associated with hypertension, coronary artery disease, and

all-cause mortality,[6-13] but the mechanisms responsible for these associations remain unclear.

Atherosclerotic disease is a ubiquitous process involving plaque formation throughout the

arterial tree. The formation of these lesions is now seen as a process of lipid deposition within

the intima of the vessel wall, oxidation of the lipoproteins, followed by endothelial activation -

resulting in leukocyte adhesion and migration into the vessel wall.[14-16] The presumed pathways

are complex, involving T cells, macrophages, neutrophils and dendritic cells, accumulation of

apoptotic cells, and ultimately to plaque formation, rupture and thrombosis.[17-19]

We suggest that urate crystals in the arterial intima of gouty patients could, in some cases,

drive the inflammation that causes plaques to rupture, and could thereby factor in their cardiac

risk. This role is well established in gouty joints where microcrystalline activation of the NALP3

inflammasome pathway induces multiple inflammatory mediators including IL-1β,[20] and leads to

gouty arthritis. In sum, MSU crystal deposits within coronary arteries could potentially trigger a

corresponding inflammatory cascade that eventuates in thrombosis and infarction.

We are aware of only one previous report of apparent urate crystals in a coronary artery.

There, Traut, et al[21] describe a man with tophaceous gout who died of a ruptured abdominal

aortic aneurysm with birefringent, spherulitic deposits in the intima of a coronary artery on

autopsy. This report preceded the application of first order red plates,[22] so it is not known

whether the crystalline deposits were negatively birefringent, as urate crystals are, or positively

birefringent, as calcium pyrophosphate crystals are.

In contrast to the rarity of arterial crystals, multiple reports of cardiac valvular tophi have been

published.[23-28] Presumably, individual MSU crystals must also deposit on the valves, though

their presence has not been described. Aortic stenosis is a common cause of valvular disease,

with calcific stenosis prevalence increasing with age to approximately 2.8% in ages ≥75 years.[29]

Aortic valve degeneration is also associated with male gender, hypertension, and metabolic

syndrome,[30,31] all risk factors associated with hyperuricemia and gout. Additionally, higher

serum uric acid levels have been described in patients with severe calcific aortic stenosis

compared to mild or moderate aortic stenosis.[32]

Lastly, chronic nonbacterial prostatitis or chronic pelvic pain syndrome (CPPS), a condition

characterized by chronic pelvic pain of unidentifiable cause for greater than three of the

preceding six months, is a common problem, affecting 2-10% of men.[33] Effective treatment

remains elusive, and the condition is frustrating to physicians as well as affected patients.

Various etiologies have been proposed, including occult infection, neurologic dysfunction,

psychological factors, and immune dysregulation.[34] The role of inflammation remains

controversial, as the presence of leukocytes on prostate biopsy does not correlate with severity

of symptoms.[35] However, molecular studies have shown increased cytokine levels in CPPS

patients, including IL-8, CXCL5 or epithelial neutrophil activating peptide 78 (ENA-78),[36] tumor

necrosis factor α (TNFα), and interestingly IL-1β.[37] In addition, a study of expressed prostatic

secretions in 56 men with chronic prostatitis found that higher urate concentrations correlated

with prostatic symptoms, though the specimens were not examined for crystals.[38] The authors

then performed a placebo-controlled trial of allopurinol at 300mg or 600mg a day in 54 men for

8 months. The allopurinol treated patients demonstrated statistically significant lower urate

levels in expressed prostatic secretions as well as improved subjective pain compared to the

placebo group.[39] To our knowledge, MSU crystals have not been sought or identified in

prostate tissue prior to our present study.

Given the evidence that tophaceous MSU deposition is widespread and that hyperuricemia now

approaches epidemic proportions, we suspected that subclinical, microscopic deposition must

also be widespread. To address this suspicion, we carried out a pilot crystal prevalence study in

3 human conditions where appropriate tissue samples were available and chronic inflammation

remains problematic: coronary disease, aortic stenosis, and chronic prostatitis.

III. METHODS

Coronary artery evaluation. Coronary arteries (left anterior descending, right coronary, left

main, and circumflex) fixed in methacarn [60% absolute methanol (Sigma-Aldrich, St. Louis,

MO), 30% chloroform (Sigma-Aldrich, St. Louis, MO), 10% glacial acetic acid (Sigma-Aldrich, St.

Louis, MO)] obtained from fifty-five explanted hearts at the time of heart transplant conducted

from 2000-2003 at the University of Washington Medical Center were examined with polarizing

microscopy (Olympus BX50, Tokyo, Japan) and photographed (Canon EOS 5D Mark II, Tokyo,

Japan). Three to six consecutive sections of each artery were examined. To be considered

positive, visualized crystals had to be present in two or more sections the same specimen.

Deconvolution microscopy (Zeiss 510 META, Oberkochen, Germany) was used to verify the

intracellular nature of the crystals in one specimen. Clinical records of these individual patients

were not available as the specimens had been de-identified as required by the University of

Washington institutional review board. Since the clinical indications for cardiac transplantation

have not changed, we carried out a demographic review of 50 consecutive patients undergoing

heart transplantation from January 2010-March 2012 (Table 1). Of these fifty patients, 19

(38%) had a history of gout and 24 (48%) were hyperuricemic (serum uric acid > 7mg/dL).

Table 1. Clinical characteristics of heart transplant patients from 2010-2012. Coronary arteries

for this study were collected from heart transplants occurring from 2000-2003.

	N	%
Gender		
Males	40	80
Females	10	20
Age (years)		
19-29	4	8
30-39	2	4
40-49	10	20
50-59	20	40
60-69	14	28
>70	0	0
Gout	19	38
Hyperuricemia (serum uric acid >7mg/dL)	24	48
Urate lowering therapy	16	32
Diuretic use	34	68
Chronic Kidney Disease	30	60
Indication for transplant		
Ischemic cardiomyopathy	19	38
Dilated cardiomyopathy	18	36
Other	13	26

Aortic valve evaluation. Seventy-five aortic valves obtained from aortic valve replacement

conducted from 2011-2013 were fixed in methacarn, decalcified in RDO acid (Apex Engineering,

Plainfield, IL), and examined with polarizing microscopy. Four consecutive sections were

examined for each valve. To be considered positive, visualized crystals had to be present in two

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or more sections of the same specimen. Clinical information for these patients was available

for review though serum urate was known for only four subjects. Eight of the 75 (10.7%)

patients had a history of gout, 6 (8%) were on allopurinol, 31 (41.3%) were on diuretics, 17

(22.7%) with renal insufficiency. Three patients (4.0%) had a SUA > 7mg/dL, and all three of

these patients also carried a diagnosis of gout.

Prostate evaluation. Forty frozen, unfixed, prostates from consecutive radical prostatectomies

performed for prostate cancer from 2012-2013 were assessed. Non-malignant regions were

evaluated with polarizing microscopy. One section from each prostate was examined. H&E

stained serial sections were examined independently for inflammatory foci in a blinded fashion.

Inflammation was graded on a scale of I-IV based on number of inflammatory foci consisting of

mononuclear infiltrates, giant cells, and/or polymorphonuclear cells. Grade I was assigned for

1-2 foci, grade II for 3-4 foci, grade III for 4-5 foci, and grade IV for 6 or more foci of

inflammatory cells within the section.

None of the patients had a recorded history of chronic prostatitis, gout, urate-lowering therapy,

or hyperuricemia.

The University of Washington Institutional Review Board approved our crystal-prevalence

study.

IV. RESULTS

Coronary artery evaluation. Of the 55 hearts examined, 6 arteries from different hearts (10.9%), contained birefringent crystals. One patient had intracellular, negatively birefringent, acicular crystals in the intima of the left anterior descending (Figure 1). One patient had a microtophus in the intima of the left anterior descending (Figure 2). One patient had negatively birefringent crystals and microtophi in the adventitia of the right coronary artery (Figure 3). Three additional patients had highly birefringent accumulations of intimal, extracellular unidentifiable crystals.

Aortic valve evaluation. Of the 75 aortic valves assessed, one valve (1.3%) had both positively and negatively birefringent crystals present scattered throughout.

Prostate evaluation. Nineteen of the forty prostates (47.5%) contained abundant, acicular (needle-shaped) birefringent crystals. The majority were negatively birefringent, though positively birefringent crystals were also seen. All crystals were present within the lumens of the glands (Figure 4).

By severity of inflammation, crystals were seen in 5 of the 12 grade I specimens, 6 of 13 grade II specimens, 4 of 6 grade III specimens, 4 of 7 grade IV specimens. Thirty-eight specimens contained mononuclear infiltrates, nine with polymorphonuclear cells, and one with giant cells. Thus, presence of crystals did not correlate with inflammatory foci grade.

V. CONCLUSIONS

When serum is hyperuricemic, so too are all other extracellular body fluids other than cerebrospinal fluid and sweat. Urate crystals are known to precipitate in loci other than the joints when supersaturated with uric acid. Anecdotal tophi have been reported in many different organs[1] though prevalence data of individual crystals are lacking. Are such deposits exceptional, or are they the tip of a crystalline iceberg?

Little data exists on the prevalence of tissue MSU crystal deposition in non-gouty patients. An autopsy study of 1733 unselected Australian patients found 8% of kidneys contained urate deposits,[40] many from individuals with no known history of gout. In classic autopsy studies of 279 gouty subjects, Talbott found normal kidneys in only four, and gross crystalline deposits in many.[41] In others, crystal presence could be ascertained by the gritty feeling when the kidneys were cut with a knife.[2] A study of cadaveric first metatarsophalangeal joints found 17% of 70 consecutive autopsies of men and women contained MSU crystals. Only 2 of the 12 patients with MSU crystals had a history of podagra or gout.[42] With the advent of newer imaging modalities such as ultrasound and dual energy computed tomography (DECT), our understanding of urate deposition continues to evolve,[3-5] but we know of no other studies of microscopic prevalence.

Our study demonstrates crystal deposition within a significant percentage of coronary arteries with one artery containing characteristic acicular, intracellular negatively birefringent crystals. In that specimen, one focus of mononuclear cells contained many with crystalline inclusions,

but no polymorphonuclear leukocytes were seen. Validation of our hypothesis, that crystal-driven inflammation may cause plaque rupture, and coronary thrombosis in some gouty patients, would seem to require intracellular crystals at the site of such a fatal lesion. To make that observation, the pathologist must have sufficient suspicion to fix the tissue in alcohol, due to formalin's formation of highly soluble addition products with urate,[43] and must then examine the resultant sections under polarized light. Neither alcohol fixation nor polarized inspection are standard procedures, but we hope that pathologists evaluating lethal myocardial infarctions will be willing to use them in search of this critically important possibility.

Confirmation of crystal composition can be difficult in tissue sections. In contrast to the thin film seen by rheumatologists in synovial aspirates, most tissue slices are ~7 microns in thickness and this is about the length of the prototypic urate crystal. Most crystals will then angle away from the tissue plane and appear to be short with varying thickness. In addition, the cytoplasmic constituents above and below a crystal of interest will lead to light scattering with indistinct crystal borders. We suspect that these issues are the principal reasons why most of the reports of crystal deposits in nonarticular locations are not illustrated by a photograph.

We further suspect that there is an as yet undefined alteration in the composition of urate deposits in some tissues. In some of the microtophi we have observed, the crystals occur not only in the classic needle and/or spherulite form but also as plates which sometimes stack upon each other. Plate-like crystals have been described in tophi in addition to classic needle-shaped crystals in a case report.[44]   Additionally, Spitz, et al in 1949 observed two morphologic types of

crystals in bony, cartilage, and renal tophi in a man with diffuse tophaceous gout.[45] Crystals

with "fine needles" in a "radial arrangement" with surrounding giant cells, lymphocytes and

fibroblasts were described. These typical crystals dissolved with formalin fixation, paraffin

embedding, and H&E staining but did not dissolve with alcohol fixation and De Galantha

staining. A second type of crystal remained after formalin fixation, paraffin embedding, H&E

staining, and decalcification with weak acids (sodium citrate and formic acid). These were

described as rhomboid, "doubly refractive," with "longitudinal striations," and oriented in

"irregular clusters or sheaves of elongated blunt needles." We have studied such deposits in

slides from the kidney of a man with severe, tophaceous gout. The slides were decalcified so

calcium crystals were unlikely. The deposits did not dissolve with topical uricase or formalin.

They did not appear to be protein-coated since applied protease did not affect their resistance

to uricase. They did stain positively with De Galantha's stain, though this stain for urate crystals

is nonspecific, and with the introduction of polarizing microscopy, this stain is no longer

regularly applied in practice.[46] We mention this experience because the long-standing

hyperuricemia and abundant tophi in this man with a long history of repeated gouty arthritis

made that diagnosis undeniable. We acknowledge that not all of the crystals we have found

must be monosodium urate monohydrate. Urates aside from monosodium urate, including

calcium urate, potassium urate, and magnesium urate, are known to form within the urinary

tract leading to renal calculi,[47,48] and could be contributing to this other type of crystal seen in

renal tophi and other tissues as well. Additionally, urate is a well-known chelator of metal ions

such as zinc, copper, and iron, and may form salts of metallo-urate complexes.[49,50] It remains

unclear whether such complexes might form birefringent crystals, though.

The great majority of the crystals we found in prostates were morphologically identical to those we expect to see in gouty joints. Although none of the prostates we examined were obtained from patients with a recognized history of gout or hyperuricemia, we believe this may not be accurate. Most of these men were referred to our urologists from outside of our hospital system, and the exact prevalence of gout and hyperuricemia may not have been reflected in the medical record.

We chose to study prostates because they are so often afflicted with chronic inflammation, and we had access to a large repository of prostate tissues. Each specimen was frozen and thus had not seen the formalin fixation that dissolves most tissue urate before the pathologist ever sees the slides. All of these specimens also contained malignant areas, but no control prostates were examined. Our data cannot be taken as indicative of a causal link between crystals and cancer.

A potential mechanism of prostatic crystal deposition could be due to reflux of urine supersaturated with uric acid. However, the crystal morphology we observed was the classic needle-shape of urate, rather than uric acid, which is generally diamond- or barrel-shaped. Irrespective of its source, the presence of so many urate crystals strongly suggests the possibility that some, much, or most prostatitis is crystal-driven. Given the highly effective armamentarium with which we now fight gout, the investigational opportunities are obvious as

is the hope that such medications may be found of use in the management of this frustrating

problem.

Only a small percentage of aortic valves in this study contained birefringent crystals; thus

reinforcing the concept that most of the apparently "tophaceous" deposits at this site are

calcific. MSU crystals are unlikely to be a significant contributor to aortic valve disease, but the

possibility continues to merit consideration in the hyperuricemic patient with tophi elsewhere.

Over the years the conception of gout has evolved away from that of a periodic nuisance that

sometimes could be damaging. Instead, it is increasingly clear that this is a progressive,

systemic disease with panoply of injurious manifestations throughout the body. We hope that

our small pilot study will encourage others to examine the epidemiology of urate deposition as

well as the consequences of such deposits throughout the body. Uric acid itself has been

recognized as a damage-associated molecular pattern (DAMP),[51,52] and there has been

significant debate about whether uric acid is pathologic or a bystander to tissue injury. In this

study we found that MSU crystals are present in coronary arteries and prostates, suggestive

that they may play a role as a trigger for the NALP3 inflammasome pathway, just as they do

within the joint. Further studies are warranted to investigate the consequences to MSU crystals

within these tissues.

Contributorship Statement: JP performed polarizing microscopy, chart review, and drafted the

manuscript. MR provided prostate pathology expertise. DS collected tissue samples and

performed polarizing microscopy. NM provided cardiac tissue samples. PS conceived and

designed the study, and drafted the manuscript. All authors read and approved the final

manuscript.

Data Sharing Statement: There are several photos of crystals within prostates that were not

included. Photos of the crystals of the one aortic valve were not included. Photos are available

by emailing janepark@uw.edu.

References:

1.   Forbess LJ, Fields TR. The broad spectrum of urate crystal deposition: unusual presentations of gouty tophi. *Semin Arthritis Rheum.* 2012;**42**:146-54.

2.   Weiss TE, Segaloff A. Gouty arthritis and gout; an ancient disease with modern interest. Springfield, Ill.,: Thomas; 1959.

3.   Dalbeth N, Kalluru R, Aati O, et al. Tendon involvement in the feet of patients with gout: a dual-energy CT study. *Ann Rheum Dis.* 2013;**72**:1545-8.

4.   Gruber M, Bodner G, Rath E, et al. Dual-energy computed tomography compared with ultrasound in the diagnosis of gout. *Rheumatology (Oxford.* 2013;**72**:1545-1548.

5.   McQueen FM, Reeves Q, Dalbeth N. New insights into an old disease: advanced imaging in the diagnosis and management of gout. *Postgrad Med J*. 2013;**89**:87-93.

6.   Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med*. 2008;**359**:1811-21.

7.   Ioachimescu AG, Brennan DM, Hoar BM, et al. Serum uric acid is an independent predictor of all-cause mortality in patients at high risk of cardiovascular disease: a preventive cardiology information system (PreCIS) database cohort study. *Arthritis Rheum.* 2008;**58**:623-30.

8.   Kuo CF, See LC, Luo SF, et al. Gout: an independent risk factor for all-cause and cardiovascular mortality. *Rheumatology (Oxford).* 2010;**49**:141-6.

9.   Moriarity JT, Folsom AR, Iribarren C, et al. Serum uric acid and risk of coronary heart disease: Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol*. 2000;**10**:136-43.

10.   Krishnan E, Pandya BJ, Chung L, et al. Hyperuricemia and the risk for subclinical coronary atherosclerosis--data from a prospective observational cohort study. *Arthritis Res Ther*. 2011;**13**:R66.

11.   Choi HK, Curhan G. Independent impact of gout on mortality and risk for coronary heart disease. *Circulation.* 2007;**116**:894-900.

12.   De Vera MA, Rahman MM, Bhole V, et al. Independent impact of gout on the risk of acute myocardial infarction among elderly women: a population-based study. *Ann Rheum Dis*. 2010;**69**:1162-4.

13.   Chen JH, Chuang SY, Chen HJ, et al. Serum uric acid level as an independent risk factor for all-cause, cardiovascular, and ischemic stroke mortality: a Chinese cohort study. *Arthritis Rheum*. 2009;**61**:225-32.

14.   Lichtman AH, Binder CJ, Tsimikas S, et al. Adaptive immunity in atherogenesis: new insights and therapeutic approaches. *J Clin Invest*. 2013;**123**:27-36.

15.   Stoll G, Bendszus M. Inflammation and atherosclerosis: novel insights into plaque formation and destabilization. *Stroke*. 2006;**37**:1923-32.

16.   Legein B, Temmerman L, Biessen EA, et al. Inflammation and immune system interactions in atherosclerosis. *Cell Mol Life Sci*. 2013;**70**:3847-69.

17.   Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell*. 2011;**145**:341-55.

18.   Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med*. 2011;**17**:1410-22.

19.   Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol.* 2011;**12**:204-12.

20.   Martinon F, Petrilli V, Mayor A, et al. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*. 2006;**440**:237-41.

21.   Traut EF, Knight AA, Szanto PB, et al. Specific vascular changes in gout. *JAMA*. 1954;**156**:591-3.

22.   McCarty DJ, Hollander JL. Identification of urate crystals in gouty synovial fluid. *Ann Intern Med*. 1961;**54**:452-60.

23.   Dennstedt FE, Weilbaecher DG. Tophaceous mitral value: Report of a case. *Am J Surg Pathol*. 1982;**6**:79-81.

24.   Jaworski RC, Gibson M. Tophaceous aortic valve: a case report. *Pathology.* 1983;**15**:197-9.

25. Curtiss EI, Miller TR, Shapiro LS. Pulmonic regurgitation due to valvular tophi. *Circulation.* 1983;**67**:699-701.

26. Scalapino JN, Edwards WD, Steckelberg JM, et al. Mitral stenosis associated with valvular tophi. *Mayo Clin Proc*. 1984;**59**:509-12.

27. Gawoski JM, Balogh K, Landis WJ. Aortic valvular tophus: identification by X-ray diffraction of urate and calcium phosphates. *J Clin Pathol*. 1985;**38**:873-6.

28. Iacobellis G. A rare and asymptomatic case of mitral valve tophus associated with severe gouty tophaceous arthritis. *J Endocrinol Invest*. 2004;**27**:965-6.

29. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation*. 2013;**127**:e6-e245.

30. Stewart BF, Siscovick D, Lind BK, et al. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. *J Am Coll Cardiol*. 1997;**29**:630-4.

31. Katz R, Wong ND, Kronmal R, et al. Features of the metabolic syndrome and diabetes mellitus as predictors of aortic valve calcification in the Multi-Ethnic Study of Atherosclerosis. *Circulation*. 2006;**113**:2113-9.

32. Demir B, Caglar IM, Ugurlucan M, et al. The relationship between severity of calcific aortic stenosis and serum uric acid levels. *Angiology.* 2012;**63**:603-8.

33. Krieger JN, Riley DE, Cheah PY, et al. Epidemiology of prostatitis: new evidence for a world-wide problem. *World J Urol*. 2003;**21**:70-4.

34. Pontari MA. Chronic prostatitis/chronic pelvic pain syndrome. *Urol Clin North Am*. 2008;**35**:81-9; vi.

35. True LD, Berger RE, Rothman I, et al. Prostate histopathology and the chronic prostatitis/chronic pelvic pain syndrome: a prospective biopsy study. *J Urol*. 1999;**162**:2014-8.

36. Hochreiter WW, Nadler RB, Koch AE, et al. Evaluation of the cytokines interleukin 8 and epithelial neutrophil activating peptide 78 as indicators of inflammation in prostatic secretions. *Urology.* 2000;**56**:1025-9.

37. Nadler RB, Koch AE, Calhoun EA, et al. IL-1beta and TNF-alpha in prostatic secretions are indicators in the evaluation of men with chronic prostatitis. *J Urol*. 2000;**164**:214-8.

38. Persson BE, Ronquist G. Evidence for a mechanistic association between nonbacterial prostatitis and levels of urate and creatinine in expressed prostatic secretion. *J Urol*. 1996;**155**:958-60.

39. Persson BE, Ronquist G, Ekblom M. Ameliorative effect of allopurinol on nonbacterial prostatitis: a parallel double-blind controlled study. *J Urol*. 1996;**155**:961-4.

40. Linnane JW, Burry AF, Emmerson BT. Urate deposits in the renal medulla. Prevalence and associations. *Nephron.* 1981;**29**:216-22.

41. Talbott JH. Gout. New York,: Grune & Stratton; 1957.

42. Wall B, Agudelo CA, Tesser JR, et al. An autopsy study of the prevalence of monosodium urate and calcium pyrophosphate dihydrate crystal deposition in first metatarsophalangeal joints. *Arthritis Rheum*. 1983;**26**:1522-4.

43. Simkin PA, Bassett JE, Lee QP. Not water, but formalin, dissolves urate crystals in tophaceous tissue samples. *J Rheum*. 1994;**21**:2320-1.

44. Sen B, Arora VK, Gupta K, et al. Platelike urate crystals in gouty tophi. *Acta Cytol*. 1993;**37**:640-1.

45. Spitz H, Steinbrocker O. Fulminating fatal gout. *Am J Med*. 1949;**6**:513-21.

46. Shidham V, Shidham G. Staining method to demonstrate urate crystals in formalin-fixed, paraffin-embedded tissue sections. *Arch Pathol Lab Med*. 2000;**124**:774-6.

47. Herring LC. Observations on the analysis of ten thousand urinary calculi. *J Urol*. 1962;**88**:545-62.

48. Bellanato J, Cifuentes JL, Salvador E, et al. Urates in uric acid renal calculi. *Int J Urol*. 2009;**16**:318-21; discussion 22.

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49.   Fleckenstein JL, Simkin PA. Zinc-mediated urate binding to human albumin. *Adv Exp Med Biol.* 1986;**195** Pt A:345-9.

50.   Mascitelli L, Pezzetta F, Goldstein MR. Comment on: Gout: an independent risk factor for all-cause and cardiovascular mortality. *Rheumatology (Oxford).* 2010;**49**:1421-2; author reply 2.

51.   Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature*. 2003;**425**:516-21.

52.   Kono H, Chen CJ, Ontiveros F, et al. Uric acid promotes an acute inflammatory response to sterile cell death in mice. *J Clin Invest*. 2010;**120**:1939-49.

Figure Legends:

Figure 1. Intracellular negatively birefringent crystals in the intima of a left anterior descending

artery (left) as seen with polarizing microscopy. White arrow denotes first-order red plate axis.

Deconvolution confocal microscopy image of the same cell showing intracellular crystals (right).

Figure 2. Microtophus in the intima of a left anterior descending artery.

Figure 3. Microtophi in the adventitia of a right coronary artery.

Figure 4. Positively and negatively birefringent crystals within prostate gland lumen.

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I. Abstract

Introduction: The prevalence of urate crystals in residual tissue samples from coronary

arteries, aortic valves and prostate glands was assessed.

Methods: Alcohol-fixed coronary arteries from 55 explanted hearts, alcohol-fixed aortic valves

collected from 75 valve replacement surgeries, and 40 frozen, unfixed prostate specimens

resected during cancer surgery were examined for birefringent crystals with polarizing

microscopy.

Results: In the 55 explanted hearts, 6 (10.9%) contained a coronary artery with birefringent

crystals. One of the 75 aortic valves (1.4%) contained both negatively and positively

birefringent crystals. 19 of the 40 (47.5%) prostates contained birefringent crystals.

Conclusion: We found that a remarkable percentage of coronary arteries and prostate

specimens contained birefringent crystals. Crystal presence is an obvious prerequisite for

possible crystal induced inflammation in these tissues, just as similar crystals elicit a gouty

inflammatory cascade in synovial joints. Further studies are necessary to determine whether

urate crystals may play this role in these tissues, and if so, to establish whether urate-lowering

therapy may be beneficial in prostatitic and coronary disease.

Strengths:

•   First study to systematically evaluate for the prevalence of urate crystals within tissues.

•   First study to show a significant percentage of extra-articular negatively birefringent

crystals.

Limitations:

• Secondary methods, aside from polarizing microscopy, to confirm urate composition of

the visualized crystals were unsuccessful.

• Clinical correlations were not possible for coronary and prostate samples since these were

taken from anonymous repositories collected for non-rheumatic diseases.

II. INTRODUCTION

By definition, hyperuricemic interstitial fluids are supersaturated, and urate supersaturation leads to precipitation of urate crystals. In joints, the crystals often drive the inflammatory disease recognized as gouty arthritis. However, hyperuricemia is not limited to plasma and the joints. All other interstitial fluids (excepting cerebrospinal fluid and sweat) will be supersaturated as well. Consequently, urate crystals will occur in non-articular tissues and may well drive crystal-induced inflammation.

In fact, anecdotal observations of visible and/or palpable tophaceous deposits have been reported from many different organs,[1] but they have usually been considered to be "medical oddities."[2] Newer imaging modalities of ultrasound and dual-energy computed tomography (DECT) have revealed far more extensive peri-articular deposition than we previously suspected.[3-5] To date, however, we have limited microscopic evidence of extra-articular urate deposition, and we do not know whether the published anecdotes are unique. As an initial venture into the field of crystal prevalence, we obtained access to unselected tissue specimens from coronary arteries, cardiac valves, and prostate glands. At each of these sites, crystal-driven inflammation could play an important role in the local pathophysiology.

Gout and hyperuricemia have been associated with hypertension, coronary artery disease, and all-cause mortality,[6-13] but the mechanisms responsible for these associations remain unclear. Atherosclerotic disease is a ubiquitous process involving plaque formation throughout the arterial tree. The formation of these lesions is now seen as a process of lipid deposition within

the intima of the vessel wall, oxidation of the lipoproteins, followed by endothelial activation - resulting in leukocyte adhesion and migration into the vessel wall.[14-16] The presumed pathways are complex, involving T cells, macrophages, neutrophils and dendritic cells, accumulation of apoptotic cells, and ultimately to plaque formation, rupture and thrombosis.[17-19]

We suggest that urate crystals in the arterial intima of gouty patients could, in some cases, drive the inflammation that causes plaques to rupture, and could thereby factor in their cardiac risk. This role is well established in gouty joints where microcrystalline activation of the NALP3 inflammasome pathway induces multiple inflammatory mediators including IL-1β,[20] and leads to gouty arthritis. In sum, MSU crystal deposits within coronary arteries could potentially trigger a corresponding inflammatory cascade that eventuates in thrombosis and infarction.

We are aware of only one previous report of apparent urate crystals in a coronary artery. There, Traut, et al[21] describe a man with tophaceous gout who died of a ruptured abdominal aortic aneurysm with birefringent, spherulitic deposits in the intima of a coronary artery on autopsy. This report preceded the application of first order red plates,[22] so it is not known whether the crystalline deposits were negatively birefringent, as urate crystals are, or positively birefringent, as calcium pyrophosphate crystals are.

In contrast to the rarity of arterial crystals, multiple reports of cardiac valvular tophi have been published.[23-28] Presumably, individual MSU crystals must also deposit on the valves, though their presence has not been described. Aortic stenosis is a common cause of valvular disease,

with calcific stenosis prevalence increasing with age to approximately 2.8% in ages ≥75 years.[29]

Aortic valve degeneration is also associated with male gender, hypertension, and metabolic

syndrome,[30,31] all risk factors associated with hyperuricemia and gout. Additionally, higher

serum uric acid levels have been described in patients with severe calcific aortic stenosis

compared to mild or moderate aortic stenosis.[32]

Lastly, chronic nonbacterial prostatitis or chronic pelvic pain syndrome (CPPS), a condition

characterized by chronic pelvic pain of unidentifiable cause for greater than three of the

preceding six months, is a common problem, affecting 2-10% of men.[33] Effective treatment

remains elusive, and the condition is frustrating to physicians as well as affected patients.

Various etiologies have been proposed, including occult infection, neurologic dysfunction,

psychological factors, and immune dysregulation.[34] The role of inflammation remains

controversial, as the presence of leukocytes on prostate biopsy does not correlate with severity

of symptoms.[35] However, molecular studies have shown increased cytokine levels in CPPS

patients, including IL-8, CXCL5 or epithelial neutrophil activating peptide 78 (ENA-78),[36] tumor

necrosis factor α (TNFα), and interestingly IL-1β.[37] In addition, a study of expressed prostatic

secretions in 56 men with chronic prostatitis found that higher urate concentrations correlated

with prostatic symptoms, though the specimens were not examined for crystals.[38] The authors

then performed a placebo-controlled trial of allopurinol at 300mg or 600mg a day in 54 men for

8 months. The allopurinol treated patients demonstrated statistically significant lower urate

levels in expressed prostatic secretions as well as improved subjective pain compared to the

placebo group.[39] To our knowledge, MSU crystals have not been sought or identified in

prostate tissue prior to our present study.

Given the evidence that tophaceous MSU deposition is widespread and that hyperuricemia now

approaches epidemic proportions, we suspected that subclinical, microscopic deposition must

also be widespread. To address this suspicion, we carried out a pilot crystal prevalence study in

3 human conditions where appropriate tissue samples were available and chronic inflammation

remains problematic: coronary disease, aortic stenosis, and chronic prostatitis.

III. METHODS

Coronary artery evaluation. Coronary arteries (left anterior descending, right coronary, left

main, and circumflex) fixed in methacarn [60% absolute methanol (Sigma-Aldrich, St. Louis,

MO), 30% chloroform (Sigma-Aldrich, St. Louis, MO), 10% glacial acetic acid (Sigma-Aldrich, St.

Louis, MO)] obtained from fifty-five explanted hearts at the time of heart transplant conducted

from 2000-2003 at the University of Washington Medical Center were examined with polarizing

microscopy (Olympus BX50, Tokyo, Japan) and photographed (Canon EOS 5D Mark II, Tokyo,

Japan). Three to six consecutive sections of each artery were examined. To be considered

positive, visualized crystals had to be present in two or more sections the same specimen.

Deconvolution microscopy (Zeiss 510 META, Oberkochen, Germany) was used to verify the

intracellular nature of the crystals in one specimen. Clinical records of these individual patients

were not available as the specimens had been de-identified as required by the University of

Washington institutional review board. Since the clinical indications for cardiac transplantation

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Prevalence of Birefringent Crystals

have not changed, we carried out a demographic review of 50 consecutive patients undergoing

heart transplantation from January 2010-March 2012 (Table 1). Of these fifty patients, 19

(38%) had a history of gout and 24 (48%) were hyperuricemic (serum uric acid > 7mg/dL).

Table 1. Clinical characteristics of heart transplant patients from 2010-2012. Coronary arteries

for this study were collected from heart transplants occurring from 2000-2003.

	N	%
Gender		
Males	40	80
Females	10	20
Age (years)		
19-29	4	8
30-39	2	4
40-49	10	20
50-59	20	40
60-69	14	28
>70	0	0
Gout	19	38
Hyperuricemia (serum uric acid >7mg/dL)	24	48
Urate lowering therapy	16	32
Diuretic use	34	68
Chronic Kidney Disease	30	60
Indication for transplant		
Ischemic cardiomyopathy	19	38
Dilated cardiomyopathy	18	36
Other	13	26

Aortic valve evaluation. Seventy-five aortic valves obtained from aortic valve replacement

conducted from 2011-2013 were fixed in methacarn, decalcified in RDO acid (Apex Engineering,

Plainfield, IL), and examined with polarizing microscopy. Four consecutive sections were

examined for each valve. To be considered positive, visualized crystals had to be present in two

or more sections of the same specimen. Clinical information for these patients was available

for review though serum urate was known for only four subjects. Eight of the 75 (10.7%)

patients had a history of gout, 6 (8%) were on allopurinol, 31 (41.3%) were on diuretics, 17

(22.7%) with renal insufficiency. Three patients (4.0%) had a SUA > 7mg/dL, and all three of

these patients also carried a diagnosis of gout.

Prostate evaluation. Forty frozen, unfixed, prostates from consecutive radical prostatectomies

performed for prostate cancer from 2012-2013 were assessed. Non-malignant regions were

evaluated with polarizing microscopy. One section from each prostate was examined. H&E

stained serial sections were examined independently for inflammatory foci in a blinded fashion.

Inflammation was graded on a scale of I-IV based on number of inflammatory foci consisting of

mononuclear infiltrates, giant cells, and/or polymorphonuclear cells. Grade I was assigned for

1-2 foci, grade II for 3-4 foci, grade III for 4-5 foci, and grade IV for 6 or more foci of

inflammatory cells within the section.

None of the patients had a recorded history of chronic prostatitis, gout, urate-lowering therapy,

or hyperuricemia.

The University of Washington Institutional Review Board approved our crystal-prevalence

study.

IV. RESULTS

Coronary artery evaluation. Of the 55 hearts examined, 6 arteries from different hearts (10.9%), contained birefringent crystals. One patient had intracellular, negatively birefringent, acicular crystals in the intima of the left anterior descending (Figure 1). One patient had a microtophus in the intima of the left anterior descending (Figure 2). One patient had negatively birefringent crystals and microtophi in the adventitia of the right coronary artery (Figure 3). Three additional patients had highly birefringent accumulations of intimal, extracellular unidentifiable crystals.

Aortic valve evaluation. Of the 75 aortic valves assessed, one valve (1.3%) had both positively and negatively birefringent crystals present scattered throughout. ~~The visualized crystals were present in two or more sections of the same specimen.~~

Prostate evaluation. Nineteen of the forty prostates (47.5%) contained abundant, acicular (needle-shaped) birefringent crystals. The majority were negatively birefringent, though positively birefringent crystals were also seen. All crystals were present within the lumens of the glands (Figure 4).

By severity of inflammation, crystals were seen in 5 of the 12 grade I specimens, 6 of 13 grade II specimens, 4 of 6 grade III specimens, 4 of 7 grade IV specimens. Thirty-eight specimens contained mononuclear infiltrates, nine with polymorphonuclear cells, and one with giant cells. Thus, presence of crystals did not correlate with inflammatory foci grade.

V. CONCLUSIONS

When serum is hyperuricemic, so too are all other extracellular body fluids other than cerebrospinal fluid and sweat. Urate crystals are known to precipitate in loci other than the joints when supersaturated with uric acid. Anecdotal tophi have been reported in many different organs[1] though prevalence data of individual crystals are lacking. Are such deposits exceptional, or are they the tip of a crystalline iceberg?

Little data exists on the prevalence of tissue MSU crystal deposition in non-gouty patients. An autopsy study of 1733 unselected Australian patients found 8% of kidneys contained urate deposits,[40] many from individuals with no known history of gout. In classic autopsy studies of 279 gouty subjects, Talbott found normal kidneys in only four, and gross crystalline deposits in many.[41] In others, crystal presence could be ascertained by the gritty feeling when the kidneys were cut with a knife.[2] A study of cadaveric first metatarsophalangeal joints found 17% of 70 consecutive autopsies of men and women contained MSU crystals. Only 2 of the 12 patients with MSU crystals had a history of podagra or gout.[42] With the advent of newer imaging modalities such as ultrasound and dual energy computed tomography (DECT), our understanding of urate deposition continues to evolve,[3-5] but we know of no other studies of microscopic prevalence.

Our study demonstrates crystal deposition within a significant percentage of coronary arteries with one artery containing characteristic acicular, intracellular negatively birefringent crystals.

In that specimen, one focus of mononuclear cells contained many with crystalline inclusions,

but no polymorphonuclear leukocytes were seen. Validation of our hypothesis, that crystal-

driven inflammation may cause plaque rupture, and coronary thrombosis in some gouty

patients, would seem to require intracellular crystals at the site of such a fatal lesion. To make

that observation, the pathologist must have sufficient suspicion to fix the tissue in alcohol, due

to formalin's formation of highly soluble addition products with urate,[43] and must then examine

the resultant sections under polarized light. Neither alcohol fixation nor polarized inspection

are standard procedures, but we hope that pathologists evaluating lethal myocardial infarctions

~~would be~~ will be willing to use them in search of this critically important possibility.

Confirmation of crystal composition can be difficult in tissue sections. In contrast to the thin

film seen by rheumatologists in synovial aspirates, most tissue slices are ~7 microns in thickness

and this is about the length of the prototypic urate crystal. Most crystals will then angle away

from the tissue plane and appear to be short with varying thickness. In addition, the

cytoplasmic constituents above and below a crystal of interest will lead to light scattering with

indistinct crystal borders. We suspect that these issues are the principal reasons why most of

the reports of crystal deposits in nonarticular locations are not illustrated by a photograph.

We further suspect that there is an as yet undefined alteration in the composition of urate

deposits in some tissues. In some of the microtophi we have observed, the crystals occur not

only in the classic needle and/or spherulite form but also as plates which sometimes stack upon

each other. Plate-like crystals have been described in tophi in addition to classic needle-shaped

crystals in a case report.[44] Additionally, Spitz, et al in 1949 observed two morphologic types of crystals in bony, cartilage, and renal tophi in a man with diffuse tophaceous gout.[45] Crystals with "fine needles" in a "radial arrangement" with surrounding giant cells, lymphocytes and fibroblasts were described. These typical crystals dissolved with formalin fixation, paraffin embedding, and H&E staining but did not dissolve with alcohol fixation and De Galantha staining. A second type of crystal remained after formalin fixation, paraffin embedding, H&E staining, and decalcification with weak acids (sodium citrate and formic acid). These were described as rhomboid, "doubly refractive," with "longitudinal striations," and oriented in "irregular clusters or sheaves of elongated blunt needles." We have studied such deposits in slides from the kidney of a man with severe, tophaceous gout. The slides were decalcified so calcium crystals were unlikely. The deposits did not dissolve with topical uricase or formalin. They did not appear to be protein-coated since applied protease did not affect their resistance to uricase. They did stain positively with De Galantha's stain, though this stain for urate crystals is nonspecific, and with the introduction of polarizing microscopy, this stain is no longer regularly applied in practice.[46] We mention this experience because the long-standing hyperuricemia and abundant tophi in this man with a long history of repeated gouty arthritis made that diagnosis undeniable. We acknowledge that not all of the crystals we have found must be monosodium urate monohydrate. Urates aside from monosodium urate, including calcium urate, potassium urate, and magnesium urate, are known to form within the urinary tract leading to renal calculi,[47,48] and could be contributing to this other type of crystal seen in renal tophi and other tissues as well. Additionally, urate is a well-known chelator of metal ions

such as zinc, copper, and iron, and may form salts of metallo-urate complexes.[49,50] It remains

unclear whether such complexes might form birefringent crystals, though.

The great majority of the crystals we found in prostates were morphologically identical to those

we expect to see in gouty joints. Although none of the prostates we examined were obtained

from patients with a recognized history of gout or hyperuricemia, we believe this may not be

accurate. Most of these men were referred to our urologists from outside of our hospital

system, and the exact prevalence of gout and hyperuricemia may not have been reflected in

the medical record.

We chose to study prostates because they are so often afflicted with chronic inflammation, and

we had access to a large repository of prostate tissues. Each specimen was frozen and thus had

not seen the formalin fixation that dissolves most tissue urate before the pathologist ever sees

the slides. All of these specimens also contained malignant areas, but no control prostates

were examined. Our data cannot be taken as indicative of a causal link between crystals and

cancer.

A potential mechanism of prostatic crystal deposition could be due to reflux of urine

supersaturated with uric acid. However, the crystal morphology we observed was the classic

needle-shape of urate, rather than uric acid, which is generally diamond- or barrel-shaped.

Irrespective of its source, the presence of so many urate crystals strongly suggests the

possibility that some, much, or most prostatitis is crystal-driven. Given the highly effective

armamentarium with which we now fight gout, the investigational opportunities are obvious as is the hope that such medications may be found of use in the management of this frustrating problem.

Only a small percentage of aortic valves in this study contained birefringent crystals; thus reinforcing the concept that most of the apparently "tophaceous" deposits at this site are calcific. MSU crystals are unlikely to be a significant contributor to aortic valve disease, but the possibility continues to merit consideration in the hyperuricemic patient with tophi elsewhere.

Over the years the conception of gout has evolved away from that of a periodic nuisance that sometimes could be damaging. Instead, it is increasingly clear that this is a progressive, systemic disease with panoply of injurious manifestations throughout the body. We hope that our small pilot study will encourage others to examine the epidemiology of urate deposition as well as the consequences of such deposits throughout the body. Uric acid itself has been recognized as a damage-associated molecular pattern (DAMP),[51,52] and there has been significant debate about whether uric acid is pathologic or a bystander to tissue injury. In this study we found that MSU crystals are present in coronary arteries and prostates, suggestive that they may play a role as a trigger for the NALP3 inflammasome pathway, just as they do within the joint. Further studies are warranted to investigate the consequences to MSU crystals within these tissues.

Data Sharing Statement: There are several photos of crystals within prostates that were not included. Photos of the crystals of the one aortic valve were not included. Photos are available by emailing janepark@uw.edu.

Figure Legends:

Figure 1. Intracellular negatively birefringent crystals in the intima of a left anterior descending artery (left) as seen with polarizing microscopy. White arrow denotes first-order red plate axis. Deconvolution confocal microscopy image of the same cell showing intracellular crystals (right).

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Figure 2. Microtophus in the intima of a left anterior descending artery.

Figure 3. Microtophi in the adventitia of a right coronary artery.

Figure 4. Positively and negatively birefringent crystals within prostate gland lumen.

References:

1. Forbess LJ, Fields TR. The broad spectrum of urate crystal deposition: unusual presentations of gouty tophi. *Semin Arthritis Rheum.* 2012;**42**:146-54.
2. Weiss TE, Segaloff A. Gouty arthritis and gout; an ancient disease with modern interest. Springfield, Ill.,: Thomas; 1959.
3. Dalbeth N, Kalluru R, Aati O, et al. Tendon involvement in the feet of patients with gout: a dual-energy CT study. *Ann Rheum Dis.* 2013;**72**:1545-8.
4. Gruber M, Bodner G, Rath E, et al. Dual-energy computed tomography compared with ultrasound in the diagnosis of gout. *Rheumatology (Oxford.* 2013;**72**:1545-1548.
5. McQueen FM, Reeves Q, Dalbeth N. New insights into an old disease: advanced imaging in the diagnosis and management of gout. *Postgrad Med J.* 2013;**89**:87-93.
6. Feig DI, Kang DH, Johnson RJ. Uric acid and cardiovascular risk. *N Engl J Med*. 2008;**359**:1811-21.
7. Ioachimescu AG, Brennan DM, Hoar BM, et al. Serum uric acid is an independent predictor of all-cause mortality in patients at high risk of cardiovascular disease: a preventive cardiology information system (PreCIS) database cohort study. *Arthritis Rheum.* 2008;**58**:623-30.
8. Kuo CF, See LC, Luo SF, et al. Gout: an independent risk factor for all-cause and cardiovascular mortality. *Rheumatology (Oxford).* 2010;**49**:141-6.
9. Moriarity JT, Folsom AR, Iribarren C, et al. Serum uric acid and risk of coronary heart disease: Atherosclerosis Risk in Communities (ARIC) Study. *Ann Epidemiol*. 2000;**10**:136-43.
10. Krishnan E, Pandya BJ, Chung L, et al. Hyperuricemia and the risk for subclinical coronary atherosclerosis--data from a prospective observational cohort study. *Arthritis Res Ther*. 2011;**13**:R66.
11. Choi HK, Curhan G. Independent impact of gout on mortality and risk for coronary heart disease. *Circulation.* 2007;**116**:894-900.
12. De Vera MA, Rahman MM, Bhole V, et al. Independent impact of gout on the risk of acute myocardial infarction among elderly women: a population-based study. *Ann Rheum Dis*. 2010;**69**:1162-4.
13. Chen JH, Chuang SY, Chen HJ, et al. Serum uric acid level as an independent risk factor for all-cause, cardiovascular, and ischemic stroke mortality: a Chinese cohort study. *Arthritis Rheum*. 2009;**61**:225-32.
14. Lichtman AH, Binder CJ, Tsimikas S, et al. Adaptive immunity in atherogenesis: new insights and therapeutic approaches. *J Clin Invest*. 2013;**123**:27-36.
15. Stoll G, Bendszus M. Inflammation and atherosclerosis: novel insights into plaque formation and destabilization. *Stroke*. 2006;**37**:1923-32.
16. Legein B, Temmerman L, Biessen EA, et al. Inflammation and immune system interactions in atherosclerosis. *Cell Mol Life Sci*. 2013;**70**:3847-69.
17. Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. *Cell*. 2011;**145**:341-55.
18. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med*. 2011;**17**:1410-22.

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Prevalence of Birefringent Crystals

19.   Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol.* 2011;**12**:204-12.

20.   Martinon F, Petrilli V, Mayor A, et al. Gout-associated uric acid crystals activate the NALP3 inflammasome. *Nature*. 2006;**440**:237-41.

21.   Traut EF, Knight AA, Szanto PB, et al. Specific vascular changes in gout. *JAMA*. 1954;**156**:591-3.

22.   McCarty DJ, Hollander JL. Identification of urate crystals in gouty synovial fluid. *Ann Intern Med*. 1961;**54**:452-60.

23.   Dennstedt FE, Weilbaecher DG. Tophaceous mitral value: Report of a case. *Am J Surg Pathol*. 1982;**6**:79-81.

24.   Jaworski RC, Gibson M. Tophaceous aortic valve: a case report. *Pathology.* 1983;**15**:197-9.

25.   Curtiss EI, Miller TR, Shapiro LS. Pulmonic regurgitation due to valvular tophi. *Circulation.* 1983;**67**:699-701.

26.   Scalapino JN, Edwards WD, Steckelberg JM, et al. Mitral stenosis associated with valvular tophi. *Mayo Clin Proc*. 1984;**59**:509-12.

27.   Gawoski JM, Balogh K, Landis WJ. Aortic valvular tophus: identification by X-ray diffraction of urate and calcium phosphates. *J Clin Pathol*. 1985;**38**:873-6.

28.   Iacobellis G. A rare and asymptomatic case of mitral valve tophus associated with severe gouty tophaceous arthritis. *J Endocrinol Invest*. 2004;**27**:965-6.

29.   Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics--2013 update: a report from the American Heart Association. *Circulation*. 2013;**127**:e6-e245.

30.   Stewart BF, Siscovick D, Lind BK, et al. Clinical factors associated with calcific aortic valve disease. Cardiovascular Health Study. *J Am Coll Cardiol*. 1997;**29**:630-4.

31.   Katz R, Wong ND, Kronmal R, et al. Features of the metabolic syndrome and diabetes mellitus as predictors of aortic valve calcification in the Multi-Ethnic Study of Atherosclerosis. *Circulation*. 2006;**113**:2113-9.

32.   Demir B, Caglar IM, Ugurlucan M, et al. The relationship between severity of calcific aortic stenosis and serum uric acid levels. *Angiology.* 2012;**63**:603-8.

33.   Krieger JN, Riley DE, Cheah PY, et al. Epidemiology of prostatitis: new evidence for a world-wide problem. *World J Urol*. 2003;**21**:70-4.

34.   Pontari MA. Chronic prostatitis/chronic pelvic pain syndrome. *Urol Clin North Am*. 2008;**35**:81-9; vi.

35.   True LD, Berger RE, Rothman I, et al. Prostate histopathology and the chronic prostatitis/chronic pelvic pain syndrome: a prospective biopsy study. *J Urol*. 1999;**162**:2014-8.

36.   Hochreiter WW, Nadler RB, Koch AE, et al. Evaluation of the cytokines interleukin 8 and epithelial neutrophil activating peptide 78 as indicators of inflammation in prostatic secretions. *Urology.* 2000;**56**:1025-9.

37.   Nadler RB, Koch AE, Calhoun EA, et al. IL-1beta and TNF-alpha in prostatic secretions are indicators in the evaluation of men with chronic prostatitis. *J Urol*. 2000;**164**:214-8.

38.   Persson BE, Ronquist G. Evidence for a mechanistic association between nonbacterial prostatitis and levels of urate and creatinine in expressed prostatic secretion. *J Urol*. 1996;**155**:958-60.

39.   Persson BE, Ronquist G, Ekblom M. Ameliorative effect of allopurinol on nonbacterial prostatitis: a parallel double-blind controlled study. *J Urol*. 1996;**155**:961-4.

40.   Linnane JW, Burry AF, Emmerson BT. Urate deposits in the renal medulla. Prevalence and associations. *Nephron.* 1981;**29**:216-22.

41.   Talbott JH. Gout. New York,: Grune & Stratton; 1957.

42.   Wall B, Agudelo CA, Tesser JR, et al. An autopsy study of the prevalence of monosodium urate and calcium pyrophosphate dihydrate crystal deposition in first metatarsophalangeal joints. *Arthritis Rheum*. 1983;**26**:1522-4.

43.   Simkin PA, Bassett JE, Lee QP. Not water, but formalin, dissolves urate crystals in tophaceous tissue samples. *J Rheum*. 1994;**21**:2320-1.

44.   Sen B, Arora VK, Gupta K, et al. Platelike urate crystals in gouty tophi. *Acta Cytol*. 1993;**37**:640-1.

45.   Spitz H, Steinbrocker O. Fulminating fatal gout. *Am J Med*. 1949;**6**:513-21.

46.   Shidham V, Shidham G. Staining method to demonstrate urate crystals in formalin-fixed, paraffin-embedded tissue sections. *Arch Pathol Lab Med*. 2000;**124**:774-6.

47.   Herring LC. Observations on the analysis of ten thousand urinary calculi. *J Urol*. 1962;**88**:545-62.

48.   Bellanato J, Cifuentes JL, Salvador E, et al. Urates in uric acid renal calculi. *Int J Urol*. 2009;**16**:318-21; discussion 22.

49.   Fleckenstein JL, Simkin PA. Zinc-mediated urate binding to human albumin. *Adv Exp Med Biol.* 1986;**195** Pt A:345-9.

50.   Mascitelli L, Pezzetta F, Goldstein MR. Comment on: Gout: an independent risk factor for all-cause and cardiovascular mortality. *Rheumatology (Oxford).* 2010;**49**:1421-2; author reply 2.

51.   Shi Y, Evans JE, Rock KL. Molecular identification of a danger signal that alerts the immune system to dying cells. *Nature*. 2003;**425**:516-21.

52.   Kono H, Chen CJ, Ontiveros F, et al. Uric acid promotes an acute inflammatory response to sterile cell death in mice. *J Clin Invest*. 2010;**120**:1939-49.
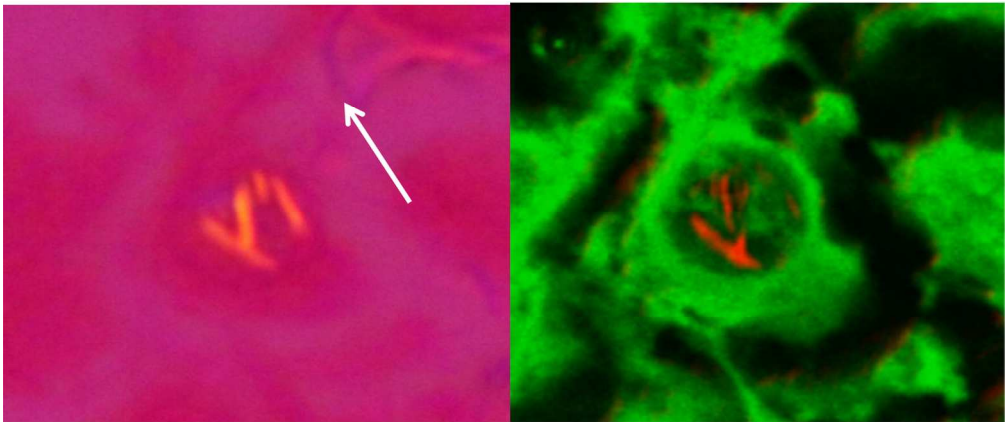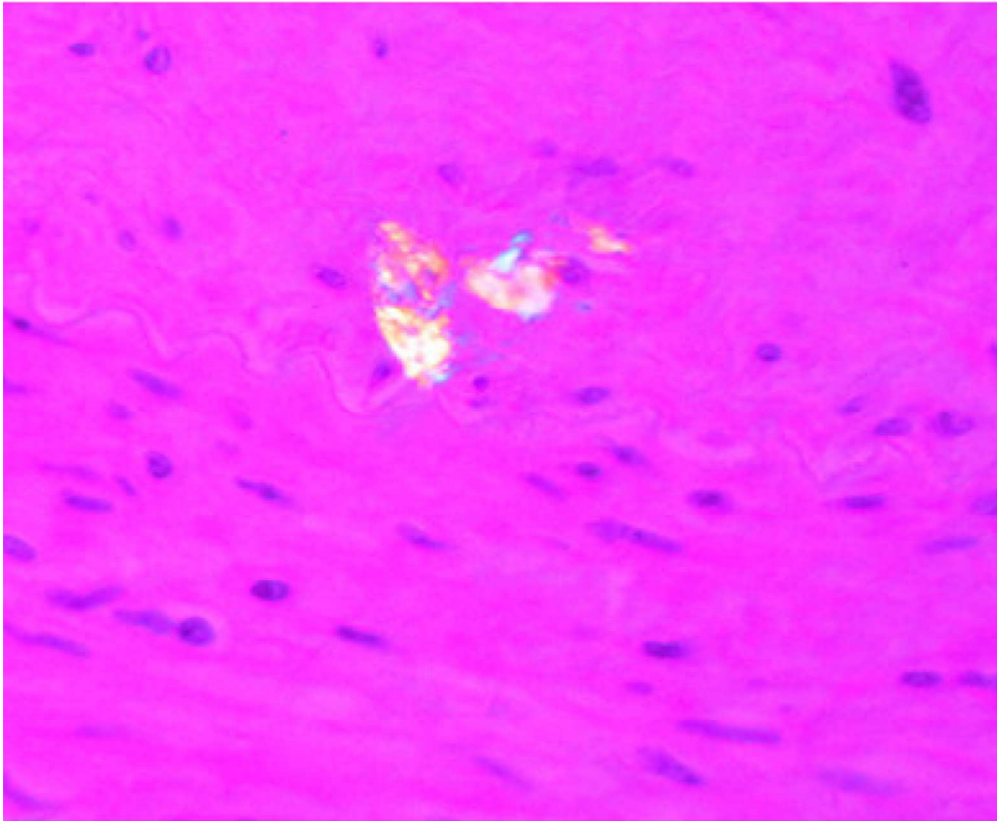
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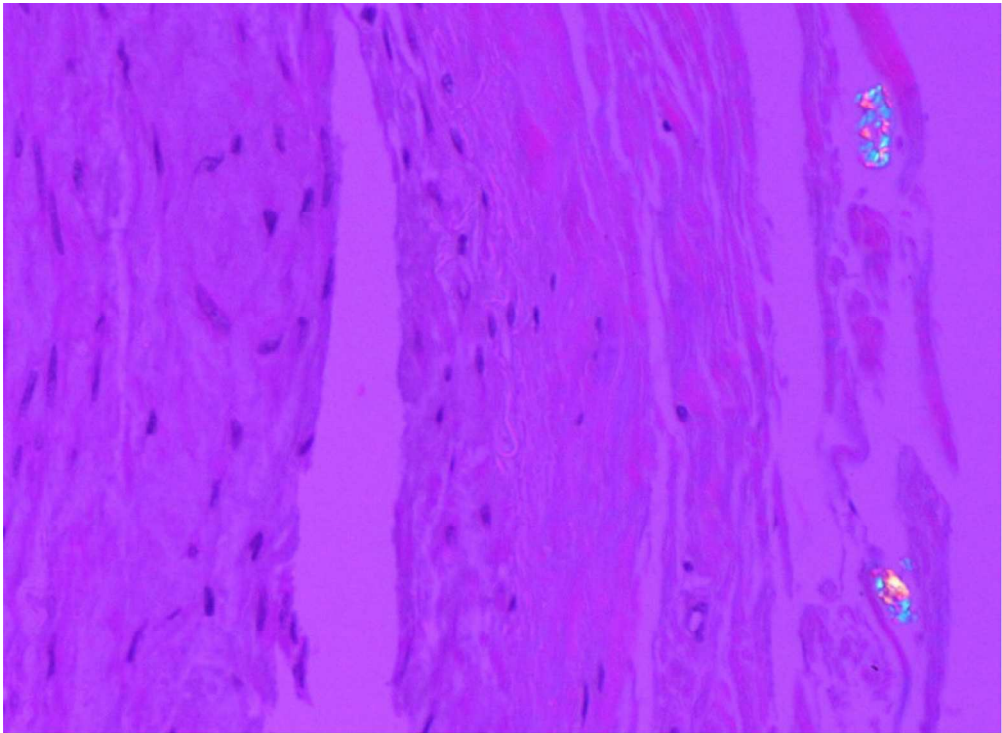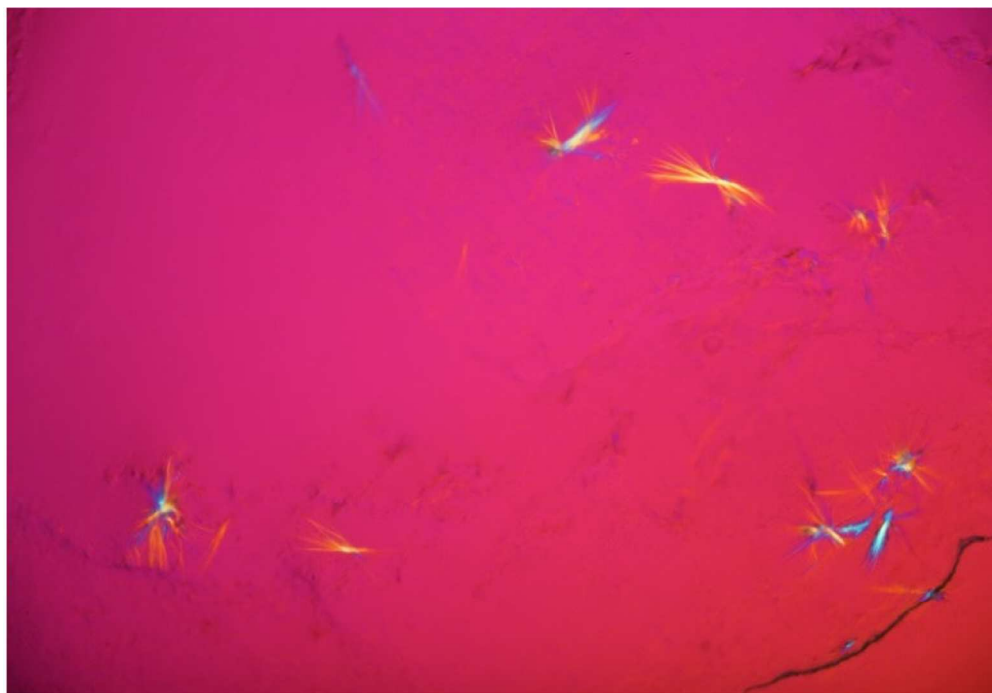159x66mm (300 x 300 DPI)

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